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Genetic and epigenetic pathways in Down syndrome: insights to the brain and immune system from humans and mouse models

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Abstract

The presence of an extra copy of human chromosome 21 (Hsa21) leads to a constellation of phenotypic manifestations in Down syndrome (DS), including prominent effects on the brain and immune system. Intensive efforts to unravel the molecular mechanisms underlying these phenotypes may help developing effective therapies, both in DS and in the general population. Here we review recent progress in genetic and epigenetic analysis of trisomy 21 (Ts21). New mouse models of DS based on syntenic conservation of segments of the mouse and human chromosomes are starting to clarify the contributions of chromosomal sub-regions and orthologous genes to specific phenotypes in DS. The expression of genes on Hsa21 is regulated by epigenetic mechanisms, and with recent findings of highly recurrent gene-specific changes in DNA methylation patterns in brain and immune system cells with Ts21, the epigenomics of DS has become an active research area. Here we highlight the value of combining human studies with mouse models for defining DS critical genes and understanding the trans-acting effects of a simple chromosomal aneuploidy on genome-wide epigenetic patterning. These genetic and epigenetic

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studies are starting to uncover fundamental biological mechanisms, leading to insights that may soon become therapeutically relevant.

Short Abstract

Here we review recent progress in genetic and epigenetic analysis of Down syndrome (DS), caused by trisomy of chromosome 21. We discuss genetic analyses using segmental human trisomy 21 (Ts21) that helped establishing more precise genotype-phenotype relationships. DS mouse models based on syntenic conservation between the mouse and human genomes have started to clarify the contributions of chromosomal sub-regions and genes to specific phenotypes in DS. The expression of human chromosome 21 genes is regulated by epigenetic mechanisms, as shown by gene-specific changes in DNA methylation patterns in brain and immune system cells with Ts21. Here we highlight the value of combining human studies with mouse models for defining DS critical genes and understanding the trans-acting effects of Ts21 on epigenetic patterns and gene regulation. These studies are starting to uncover fundamental biological mechanisms, leading to insights that may soon become therapeutically relevant.

Keywords

Trisomy 21; Down syndrome; genetics; epigenetics; animal models

Introduction

For this chapter we have merged and substantially updated two of our prior reviews on genetic and epigenetic pathways in human Down syndrome (DS) and mouse models of Ts21 (Xing *et al.* 2016; Do *et al.* 2017b). The classification of DS (Down 1887) and later the discovery of human Ts21 in cells isolated from individuals with this syndrome (LeJeune *et al.* 1959) are milestones in medical genetics. Subsequent studies focused on identifying individuals carrying partial Ts21 to establish genotype-phenotype relationships. These decades-long efforts led to the definition of sub-chromosomal regions that are associated with important medical conditions in DS. In parallel to human genetic analysis, mouse-based genetic modeling and analysis of DS was started about three decades ago, with human and mouse genome mapping and sequencing providing the evolutionary support for this work: genomic regions on human chromosome 21 (Hsa21) are syntenically conserved with three regions in the mouse genome, located on mouse chromosome 10 (Mmu10), Mmu16 and Mmu17. The mouse-based studies were markedly expedited by development of Cre/loxP-mediated chromosome engineering. By manipulating the dosage of specific Hsa21 syntenic regions, many mouse models of segmental trisomies and monosomies of these chromosomal regions have been generated, including a mouse line carrying triplications of all three Hsa21 syntenic regions. Moreover, with the support of the International Knockout Mouse Project, null alleles have now been engineered for almost all Hsa21 gene orthologs. By using individual and compound mouse mutants, the impacts of the actions of individual Hsa21 gene orthologs and contiguous groups of these gene orthologs are now being revealed. Here we review these genetic experiments, and we illustrate and discuss recent findings from combined genetic-epigenetic studies, which reveal highly recurrent and reproducible effects of the extra chromosome 21 on DNA methylation patterns at specific

sets of loci distributed across the entire genome. These differential methylation patterns (abbreviated here as DS-DM) have both tissue-specific and pan-tissue components, and we have found that, within a given tissue such as brain, the patterns of DS-DM are at least partly conserved in both chromosomally engineered and radiation-induced mouse models of DS. While more work needs to be done, these studies on the trans-acting epigenetic effects of a simple chromosomal trisomy are already starting to yield unique insights to the mechanisms by which DNA methylation patterns are established in normal and aneuploid mammalian cells. We discuss questions for future investigation, and we argue that results from genetic and epigenetic analysis of DS could have therapeutic implications, not only in DS but also in other situations such as human subchromosomal duplication and deletion syndromes, and cancers.

Genetic dissection of Down syndrome based on human partial (segmental) Ts21

Most patients with DS (about 95%) carry a whole extra chromosome 21, whereas about 4% of DS cases are partial Ts21 caused by interchromosomal or intrachromosomal rearrangements (Nadal *et al.* 2001). So far, around 200 partial Ts21 cases have been reported since the first “incomplete trisomy” was discovered (Ilbery *et al.* 1961; Pelleri *et al.* 2016). A major goal of genetic studies in DS is to define sub-genomic regions associated with various DS phenotypes by examining human segmental trisomies. In these efforts, data generated from individuals with segmental trisomy of Hsa21 were used to establish genotype-phenotype relationships (Yamada *et al.* 1998; Fotaki *et al.* 2002; Adams *et al.* 2004). There have been some interesting developments in this area (Lyle *et al.* 2009; Pelleri *et al.* 2016). After systematic analysis of the partial Ts21 cases reported from 1973 to 2015, Pelleri *et al.* built a chromosomal region map with triplicated segments from a total of about 125 cases associated with human partial Ts21. Among them, 88 individuals carrying partial Ts21 exhibited partial or complete DS phenotypes, while 37 showed no DS phenotypes and were therefore informative for excluding some chromosomal regions from the so-called “DS critical region” of Hsa21. Copy-number variants (CNV)-associated duplications were also included. According to these authors, their study led to establishing a highly restricted critical region (which they refer to as HR-DSCR) comprising only 34 kb of DNA on distal chromosome band 21q22.13, whose duplication is shared by all DS cases and is absent in all non-DS cases. This is a provocative finding. If one simply looks at the altered genomic regions associated with Hsa21, some partial Ts21 cases may not be consistent with the conclusion. For example, in Cases JG and JL shown in Figure 1, the triplications of Hsa21 regions did not contain the HR-DSCR, but the individuals still showed DS phenotypes. However, these and other similar cases cannot be used to argue against the presence of HR-DSCR because they also carry genomic alterations beyond the partial Ts21 (Korbel *et al.* 2009), which may have caused the clinical phenotypes. The 34-kb HR-DSCR DNA segment contains no protein- or miRNA-coding information as of yet, so its biological significance will need to be ascertained, for example by generating and analyzing mouse models carrying engineered duplications of this small region. This and other studies show that analysis of partial human Ts21 remains important for understanding the genetic mechanisms underlying DS phenotypes. However, interpretations of these studies are often complex for the following reasons: (1) Some individuals with partial Ts21 also carry additional genomic abnormalities, such as unbalanced derivatives associated with non-Hsa21 genomic regions

stemming from chromosomal translocations (Yamada *et al.* 1998; Fotaki *et al.* 2002; Adams *et al.* 2004). (2) The endpoints of partial trisomies are almost always unique among the cases. Therefore, for almost any segmental trisomy case, the sample size for a specific genotype is one, which is a major obstacle in distinguishing the contributions of trisomy versus unique characteristics of a given subject to a phenotype (Kahlem *et al.* 2004). For these reasons, molecular genetic dissection of DS is being complemented by generating and analyzing well-controlled animal models.

Genetic dissection of Down syndrome using animal models

The DNA sequences of human chromosome 21 (Hsa21) are syntenically conserved with three regions in the mouse genome, located on mouse chromosome 10 (Mmu10), Mmu16, and Mmu17. These shared syntenies provide an evolutionary basis for genetic modeling and dissection of DS. The Ts65Dn model, which for many years was the model of choice for DS, is trisomic for the Hsa21 syntenic region distal to *Mir155* on Mmu16, but is also trisomic for a subcentromeric region on Mmu17, which is not syntenic to Hsa21 (Davisson *et al.* 1990). Tc1 is another interesting mouse model which carries a Hsa21 (O'Doherty *et al.* 2005). Because Hsa21 was irradiated during the experiment in generating this transchromosomal model, the Hsa21 in Tc1 mice carries several genetic rearrangements, including deletions and duplications. Despite these complexities, these two models have been extensively characterized and show several phenotypic features similar to human DS (Kahlem *et al.* 2004). In the last 15 years, a significant number of new mouse mutants carrying rearranged Hsa21 syntenic regions has been developed using Cre/loxP-mediated chromosome engineering. These models include one that carries triplications spanning the three Hsa21 syntenic regions in their entirety, i.e., *Dp(10)1Yey/+;Dp(16)1Yey/+;Dp(17)1Yey/+* (Yu *et al.* 2010). Phenotyping results showed that this compound model exhibits major DS phenotypes, including cardiovascular defects, learning deficits, and motor dysfunction (Xing *et al.* 2016; Herault *et al.* 2017; Watson-Scales *et al.* 2018). Other engineered mouse mutants, which carry a duplication or deletion of smaller Hsa21 syntenic regions (Xing *et al.* 2016; Herault *et al.* 2017), are facilitating genetic dissections of DS phenotypes.

The availability of mouse models of DS carrying triplications of different Hsa21 syntenic regions (Herault *et al.* 2017) and the null alleles of individual Hsa21 gene orthologs has also made feasible to better understand the gene actions and interactions which contribute to DS phenotypes. Such a feasibility is well illustrated in the analysis of *Dyrk1a* and *Kcnj6*, which are among the major candidate genes for DS-associated cognitive deficits (Belichenko *et al.* 2009). *Dyrk1a* codes for a dual-specificity tyrosine-phosphorylation-regulated kinase which plays a critical role in signaling (Kelly and Rahmani 2005; Fernandez-Martinez *et al.* 2009; Hammerle *et al.* 2011). *Kcnj6* encodes for a G protein-activated potassium channel, which regulates neuronal excitability by mediating the inhibitory effects of G-protein-coupled receptors for neuromodulators and neurotransmitters (Yamada *et al.* 1998; Mark and Herlitz 2000). To analyze the impact of *Dyrk1a* on DS-associated cognitively-relevant phenotypes, we first generated a *Dyrk1a* mouse mutant (*Dyrk1a*^{+/-}) and then compared *Dp(16)1Yey/Dyrk1a*⁻ mice with *Dp(16)1Yey*⁺ mice and wild-type controls. Our results showed that *Dp(16)1Yey/Dyrk1a*⁻ mice performed significantly better than *Dp(16)1Yey*⁺ mice in the

cognitive behavioral tests, even though their performance did not reach the level achieved by wild-type controls statistically. When we compared hippocampal long-term potentiation (LTP), a neurophysiological phenotype associated with memory formation, we found that the magnitude of LTP after theta burst stimulation (TBS) of the brain slices from *Dp(16)1Yey/Dyrk1a*- mice was significantly higher than that from *Dp(16)1Yey/+* mice, but it was not as significant as the one from the wild-type controls. The difference in cognitive impairment between *Dp(16)1Yey* and *Dp(16)1Yey/Dyrk1a*- is due to the dosage normalization of *Dyrk1a*. Thus, *Dyrk1a* is indeed a causative gene for the cognitive deficits observed in *Dp(16)1Yey/+* mice (Jiang *et al.* 2015), but the remaining partial cognitive impairment in *Dp(16)1Yey/Dyrk1a*- mice must be caused by the triplication of a gene or genes located outside of the *Cbr1-Fam3b* region (Fig. 2, Panels A, B)

To examine the joint impact of *Dyrk1a* and *Kcnj6*, *Df(16)Dyrk1a-Kcnj6* Yey, i.e., *Df(16)7Yey* mice, were engineered. The deletion was transmitted at a ratio lower than predicted, which is likely contributed by the heterozygous deletion of *Dyrk1a*. To examine the simultaneous effect of *Dyrk1a* and *Kcnj6* on cognitively-relevant phenotypes, we compared *Dp(16)1Yey/Df(16)7Yey*, *Dp(16)1Yey/+* and wild-type control mice in the cognitive behavioral tests. Our results showed that *Dp(16)1Yey/Df(16)7Yey* mice performed significantly worse than wild-type controls and did not perform better than *Dp(16)1Yey/+* mice (Fig. 2, Panel B). Similar effects due to the genotype were observed in hippocampal LTP (Jiang *et al.* 2015).

We therefore hypothesized a novel causative gene “X” for DS-associated cognitive deficits in the *Kcnj6-Mx2* region, and that the effect of triplication of “X” was suppressed by the *Kcnj6* triplication. *Dp(16)1Yey;Ms1Rhr* mice showed a partial rescue of cognitive deficits because *Dyrk1a* was converted back to two copies. The remaining cognitive deficits are caused by the triplication of a gene or genes located outside of the *Cbr1-Fam3b* region, similarly to *Dp(16)1/Dyrk1a*- mice. In *Dp(16)1Yey;Ms1Rhr* mice, *Kcnj6* is present only in two copies so it will not inhibit the impact of the “X” triplication, but “X” is only present in two copies here so it does not contribute to cognitive deficits (Fig. 2, Panel C). In *Dp(16)1Yey/Df(16)7Yey* mice, both *Dyrk1a* and *Kcnj6* are converted to two copies so they do not contribute to cognitive deficits themselves but the “X” gene is present in three copies and contributes to cognitive deficits in *Dp(16)1Yey/Df(16)7Yey* mice because the effect of the triplication of “X” is not suppressed by the triplicated *Kcnj6* (Fig. 2, Panel D). Recently, a new study showed that converting *Kcnj6* from three copies to two copies in Ts65Dn mice also resulted in partial rescue of cognitive deficits (Kleschevnikov *et al.* 2017). This result suggests that like *Kcnj6*, *Dyrk1a* may also inhibit the “X” gene’s impact when *Kcnj6* is converted from three copies to two copies (Fig. 2, Panel E).

The next logical step is to identify the “X” gene to facilitate unraveling of the mechanism of *Kcnj6*- and *Dyrk1a*-associated suppression. Among Hsa21 gene orthologs in the *Kcnj6-Mx2* region, seven genes—*Ets2*, *Psmg1*, *Brwd1*, *Hmgn1*, *Wrb*, *Pcp4*, and *Dscam*—are possible candidates because expressed in the central nervous system (Egeo *et al.* 1998; Yamakawa *et al.* 1998; Vidal-Taboada *et al.* 2000; Barlow *et al.* 2001; Wolvetang *et al.* 2003a; Kahlem *et al.* 2004; Lyle *et al.* 2004; Song *et al.* 2008; Abuhatzira *et al.* 2011; Ling *et al.* 2014; Renelt *et al.* 2014). To test this hypothesis, it could be useful to compound a null allele of a

candidate gene with *Dp(16)1Yey* and *Df(16)7Yey* simultaneously. This is feasible because *Df(16)7Yey* could be transferred to the chromosome homolog carrying *Dp(16)1Yey* via crossover. The “X” gene could be identified by partial rescue of cognitive deficits in the triple compound mutant mice. Overall, these genetic experiments highlight an additional role for gene-gene interactions in cognitive phenotypes of DS.

As aforementioned, the mouse models developed using Cre/loxP-mediated ES cell-based chromosome engineering have played an important role in DS research. However, Cre/loxP-mediated chromosome engineering requires multistep manipulation in mouse embryonic stem cells (Yu and Bradley 2001). In addition, the most popular ES cell-based chromosome engineering procedure requires the use of ES cells carrying a null allele of *Hprt*, which are only available for a limited number of mouse strains and are not available for other species, including rats. The development of CRISPR/Cas9 genome editing may overcome these limits, for example by improving the efficiency of chromosome engineering in Hsa21 syntenic regions (Kraft *et al.* 2015; Lagutina *et al.* 2015; Boroviak *et al.* 2016; Birling *et al.* 2017). Thus, CRISPR/Cas9 will likely start to play an important role in mammalian chromosome engineering, complementing current approaches. Specifically, it may offer opportunities to obtain mutants for other less commonly used animal models by direct zygote delivery of the CRISPR/Cas9 components (Wang *et al.* 2013; Yang *et al.* 2013; Niu *et al.* 2014). This new approach may also facilitate direct chromosome engineering in mice with different strain backgrounds, which is not feasible using Cre/loxP-mediated methods (Boroviak *et al.* 2016; Birling *et al.* 2017).

In Cre/loxP-mediated ES cell-based chromosome engineering, the size of the rearranged genomic regions is inversely correlated with the efficiency of generation of the chromosomal rearrangements (Zheng *et al.* 2000), while the impact of the size of the rearranged chromosomal region has not yet fully been established in CRISPR/Cas9-mediated genome manipulation (Kraft *et al.* 2015; Boroviak *et al.* 2016; Birling *et al.* 2017; Xing *et al.* 2018). However, the genomic locations of the endpoints of the rearranged segments manipulated via CRISPR/Cas9 can affect the efficiency (Kraft *et al.* 2015). Although chromosomal rearrangements may be efficiently generated by CRISPR/Cas9, identifying them by genotyping can sometimes be challenging, with PCR failing to reveal full information on the chromosome rearrangements. A recent study suggests genomic rearrangements engineered by CRISPR/Cas9 may need more careful examination and characterization, when compared with Cre/loxP-mediated genome engineering. By analyzing mouse mutants generated via guide RNAs targeted at two endpoints separated by about 1-Mb by high-resolution fibre-FISH, Boroviak *et al.* show that the resulting rearrangements actually include duplication, inversion and complex rearrangements with multiple genomic rearrangements (Fig. 3A) (Boroviak *et al.* 2017). Therefore, genomic rearrangements generated by CRISPR/Cas9 will require extensive molecular characterization.

Because procedures using CRISPR/Cas9-mediated genome editing are changing rapidly, any major advances in utilizing this endonuclease will have impacts on modeling DS. One of the critical developments recently is the delivery of CRISPR/Cas9 reagents to two-cell-stage of mouse embryos instead of zygotes when CRISPR/Cas9 is used to generate targeted knock-ins. Two-cell-stage embryos have more open chromatin and a longer G2 phase, which

facilitates homology-directed repair and thus significantly enhances the efficiency of targeted knock-ins (Fig. 3B) (Gu *et al.* 2018).

The availability of CRISPR/Cas9-mediated genome editing technology and its rapid technical evolution open unprecedented opportunities for genetic modeling and analysis of DS in other organisms. One such area that holds great potential is modeling in non-human primates (Jennings *et al.* 2016). Two primate species, the common marmoset and the mouse lemur, have several advantages as candidates for creating transgenic models for human genetic conditions, such as DS (Sasaki *et al.* 2009). Non-human primates are the closest evolutionary relatives to humans. When compared with other non-human primates, these two species are smaller in size and have shorter lifespans, which is advantageous for laboratory experiments. Moreover, like humans, with advancing age they show Alzheimer's-type neurodegeneration (Bons *et al.* 2006; Tardif *et al.* 2011; Languille *et al.* 2012).

Epigenetic alterations in brain and immune system cells with Ts21

The most obvious hypothesis for DS pathogenesis is that it is accounted for by gene dosage effects, with increased expression of specific genes or groups of genes on HSA21 accounting for the multiple phenotypes. In fact, transcriptional levels of many HSA21 genes roughly parallel the 1.5X increase in gene copy number in human cells and tissues with Ts21, and similar dosage effects are seen in mouse models (Mao *et al.* 2003; Saran *et al.* 2003; Kahlem *et al.* 2004; Lyle *et al.* 2004; Vacik *et al.* 2005; Li *et al.* 2006; Prandini *et al.* 2007; Costa *et al.* 2011). However, our early study of gene expression in Ts21 versus control fibroblasts and fetal hearts found evidence for strong (i.e. non-linear) overexpression of a small number of genes on Hsa21 (e.g. the interferon target gene *MX1*), while conversely some other Hsa21 genes lacked detectable increases in expression compared to the euploid control samples (Li *et al.* 2006). Similar findings of a few genes that have >1.5X increased expression have been made in the other gene expression profiling studies of Ts21 referenced above. Non-linear over-expression can be explained by gene-gene interactions when multiple genes in one biological pathway reside on Hsa21. Examples are multiple HSA21 genes in the interferon pathway, which can explain the strong over-expression interferon target genes (Li *et al.* 2006; Sullivan *et al.* 2016), and an interaction between the HSA21-encoded ETS2 transcription factor and the HSA21-encoded *APP* gene, leading to non-linear overexpression of *APP* mRNA (Wolvetang *et al.* 2003b). Importantly, all genome-wide studies have shown that Ts21 produces significant changes in the expression of genes on the other (non-trisomic) chromosomes, indicating perturbations of downstream transcriptional networks.

Likely contributing to these perturbed networks, or compensating for them, studies carried out over the last 10 years have suggested that DS pathogenesis involves recurrent and reproducible epigenetic changes in the DNA and chromatin of cells with Ts21. In our initial work, microarray-based DNA methylation profiling (Illumina 27K) in peripheral blood leukocytes (PBL) from individuals with DS and age-matched controls revealed gains and losses of DNA methylation, strongly affecting ~100 genes, as a consistent epigenetic response to Ts21 in these cells (Kerkel *et al.* 2010). The set of loci with differential methylation in DS versus controls (abbreviated here as DS-DM) was not enriched for Hsa21

genes; rather, genes with DS-DM were found to be roughly evenly distributed on all chromosomes. Many research groups have built on these findings such that, as of 2019, PubMed searches for “Down syndrome and epigenetic” and “Down syndrome and methylation” each retrieved more than 100 publications. Most of these studies, including work from our group, have utilized genome-wide epigenomic profiling on denser 450K methylation BeadArrays, sometimes with validations by bisulfite sequencing, to characterize CpG methylation patterns in PBL and T lymphocytes, brain cells and whole brain tissues, and placentas, to tissue- and age-matched euploid controls (Yuen *et al.* 2011; Eckmann-Scholz *et al.* 2012; Jin *et al.* 2013; Jones *et al.* 2013; Bacalini *et al.* 2015; Horvath *et al.* 2015; Mendioroz *et al.* 2015; Sailani *et al.* 2015; Alves da Silva *et al.* 2016; El Hajj *et al.* 2016; Hatt *et al.* 2016; Lim *et al.* 2016; Henneman *et al.* 2018). The patterns of differential methylation between DS and controls in each tissue or cell type are strong and recurrent across multiple individuals with DS (Figure 4). However, except for an interesting small group of “pan-tissue” DS-DM loci, the sets of differentially methylated regions (DMRs) are largely tissue-specific (Mendioroz *et al.* 2015; Do *et al.* 2017b). Importantly, studies of DS versus control blood cells, carried out by separate laboratories in independent series of individuals from different countries (U.S.A., Italy, and the Netherlands), show substantial overlaps (Do *et al.* 2017b; Henneman *et al.* 2018). Thus, DS-DM is a well-defined and highly reproducible epigenetic response to the extra Hsa21 in DS.

Data from multiple laboratories indicate that brain cerebral cortex (and some other organs such as placenta) shows significantly more *hyper*-than *hypo*-methylated DS-DM loci, while blood cells show somewhat more *hypo*-methylated DS-DM regions (Jin *et al.* 2013; Bacalini *et al.* 2015; Mendioroz *et al.* 2015; El Hajj *et al.* 2016; Henneman *et al.* 2018). Even within the brain there are regional variations; with the cerebral cortex showing a greater bias toward hyper-methylation than the cerebellar cortex (Mendioroz *et al.* 2015). Indeed, since the patterns of DS-DM are cell type-specific, steps to separate neurons from non-neuronal cells are necessary for a complete description of this phenomenon in the brain. In our work (Mendioroz *et al.* 2015) we purified neurons from DS and control brains using the FANS procedure (Matevossian and Akbarian 2008), which entails NeuN staining of cell nuclei, followed by fluorescence-activated nuclear sorting. Data from Illumina 450K arrays, with validations by bisulfite sequencing, revealed a cell type-specific epigenetic response to Ts21 in neurons and glial cells, with only partial overlap of the sets of DS-DM loci between these cell types (Mendioroz *et al.* 2015). Oxidative Bis-Seq for the doubly-modified “sixth base”, 5hydroxymethyl-C (5hmC), showed changes in this epigenetic mark that often, though not always, paralleled DS-DM in the more extensively studied “fifth base”, 5mC (Mendioroz *et al.* 2015).

In addition to cytosine methylation and hydroxymethylation, a second major type of epigenetic marks is post-translational histone modifications. A complete description of epigenomic alterations thus should include information on both types of marks. Letourneau *et al.* assessed perturbations of gene expression in Ts21 versus normal fetal fibroblasts and found differential expression organized in broad domains along all chromosomes, with the H3K4me3 profile (a mark for transcriptionally active promoter regions) of the trisomic fibroblasts following the broad domain patterns (Letourneau *et al.* 2014). How this and other types of histone marks might correlate with and interact mechanistically with DS-DM is an

interesting question for ongoing research. It is already clear that the relationship between these two types of epigenetic marks is not simple, since H3K4me3 alterations were seen in broad chromatin domains spanning multiple genes, while DS-DM localizes to discrete regulatory regions of single genes, without obvious domain-like effects. Even when multiple adjacent genes are affected, as in the protocadherin gene cluster and other examples, the DS-DM is focal, affecting only specific types of regulatory sequences (Mendioroz *et al.* 2015; Do *et al.* 2017b). Lastly, to achieve a complete epigenomic analysis, other potential epigenetic effects, such as altered occupancy of chromatin by long non-coding RNAs (lncRNAs), will need to be assayed in cells with Ts21.

Epigenetic mechanisms revealed by studying DS-DM in humans and mouse models

Two different scenarios for trans-acting molecular mechanisms, namely a role for increased dosage of Hsa21-linked S-adenosylmethionine (SAM)-dependent methylation pathway genes and a role for increased dosage of Hsa21-linked transcription factor (TF) genes, have been postulated to underlie DS-DM. Regarding the “methylation pathway hypothesis”, Hsa21 contains a group of genes that have known or predicted roles in the biochemistry of DNA methylation (Pogribna *et al.* 2001; Blehaut *et al.* 2010; Dekker *et al.* 2014). The pathway leading to methylation of cytosines in genomic DNA requires donation of methyl groups from SAM, which in turn depends on folate metabolism in the cell. The *SCL19A1* gene codes for the reduced folate carrier protein that acts as a transporter at the plasma membrane, while the products of the *SOD1*, *GART* and *CBS* genes act in metabolic pathways involving folic acid and homocysteine in the cytoplasm. Indeed, Pogribna *et al.* have presented evidence that the decreased availability of homocysteine caused by cystathionine β -synthase (CBS) over-expression in proliferating cells with Ts21 promotes a functional folate deficiency – which could account for a mild but significant global DNA *hypo*-methylation in blood cells from individuals with DS (Pogribna *et al.* 2001). The majority of DS-DM loci in blood cells do in fact show *hypo*-methylation, but since most DS-DM loci in cerebrum and placenta predominantly show *hyper*-methylation, not *hypo*-methylation, a role for a functional folate deficiency in producing DS-DM in those tissues seems doubtful.

The *N6AMT1* and *DNMT3L* genes encode enzymes or components of enzymes that act as methyltransferases, either for cytosines in DNA (*DNMT3L*, which codes for a protein that heterodimerizes with DNMT3A and DNMT3B to form catalytically active DNA methyltransferases) or for other cellular components that could compete for methyl groups from SAM (*N6AMT1*). Lastly, the protein coded by the Hsa21 *MIS18A* gene interacts with DNMT3A/3B and has been shown to be critical for maintaining DNA methylation at centromeres (Kim *et al.* 2012). The increased dosage of these genes could in principle affect DNA methylation in cells with Ts21. Certainly, increased expression of *DNMT3L* must be considered as a possible explanation for the predominance of *hyper*-methylated DS-DM loci in placenta and fetal and adult cerebrum. Mouse models will be useful for systematically testing these ideas, since the various mouse lines carry different duplicated chromosomal regions, each with only a subset of the methylation pathway genes. For example, *DNMT3L* maps to the distal q-arm of Hsa21 and its orthologue is therefore present in three copies in the *Dp(10)Yey* mice but only in the normal two copies in the *Dp(16)Yey* and Ts65Dn

lines (Figure 5). Given the important developmental role of this gene (Bourc'his *et al.* 2001; Ooi *et al.* 2007), its known co-function and co-regulation with DNMT3B (Sailani *et al.* 2015), and the known onset of the DS-DM phenomenon early in development (e.g. in fetal brain and placenta), experiments using genetic methods to increase and decrease its copy number in mouse models and testing for phenocopying and rescue of the DS-DM, respectively, are a high priority.

To lay the groundwork for genetically dissecting the Hsa21 “effector genes” that lead to DS-DM, we carried out whole genome bisulfite sequencing (WGBS; methyl-seq) in brain tissue from three of the mouse models. First, we analyzed newborn cerebral hemispheres from *Dp(16)1Yey* and *Dp(10)1Yey* mice, which are triplicated for 115 and 41 Hsa21 gene orthologs on Mmu16 and Mmu10, respectively. The two models have different patterns of DM, but both show statistically significant similarities with human DS-DM, with a trend toward hypermethylation of the DM genes, albeit stronger in *Dp(10)1Yey* than in *Dp(16)1Yey* (Mendioroz *et al.* 2015). In both models the bias toward hypermethylation, if present, was less strong than that seen in the human 450K Beadchip data, perhaps because WGBS covers the whole methylome while the Beadchips query mostly CG-rich sequence elements. The finding of greater hypermethylation in *Dp(10)1Yey* made sense, since both *Dnmt3l* and *Slc19a1*, a Hsa21-linked methyl donor pathway gene, map to the Mmu10 duplicated region. However, since numerous DM loci, some *hyper*- and some *hypo*-methylated, were also detected in the *Dp(16)1Yey* brain, we concluded that multiple genes on Hsa21, mapping both to the *Dp(10)1Yey* and to the *Dp(16)1Yey* syntenic regions, play a role in the net phenomenon (Mendioroz *et al.* 2015). In a more recent WGBS experiment we examined cerebral cortical grey matter from Ts65Dn newborns compared to control littermates and obtained results that are again significantly similar to human DS and, as expected based on the duplicated regions, are more similar to the findings in *Dp(16)1Yey* than *Dp(10)1Yey* (Do *et al.* 2017b). As an example, the partial but statistically significant similarities between DS-DM in humans and DM in the *Dp(16)1Yey* and Ts65Dn mouse models are highlighted by high-resolution methyl-seq data for the *PCDHA-PCDHG* protocadherin gene cluster (Fig. 6).

The “TF gene over-expression and binding site occupancy” hypothesis (Fig. 5), which postulates a role for increased Hsa21-linked TF gene expression, followed by increased occupancies and protection from methylation of the specific binding sites that are recognized by these factors on various chromosomes, is supported by bioinformatic enrichment analyses in the human data. For example, we found strong enrichment of RUNX DNA binding sites among DS-DM loci in DS vs control T lymphocytes (Mendioroz *et al.* 2015; Do *et al.* 2017b). The *RUNX1* gene maps to Hsa21 and, despite partial gains of CpG methylation in its promoter/enhancer region (which shows DS-DM in both T cells and brain cells), *RUNX1* mRNA is modestly over-expressed in DS lymphocytes. So, the simplest interpretation for the bioinformatic enrichment data is that the over-expressed RUNX1 protein is occupying its cognate DNA binding sites on various chromosomes to a greater extent, thereby blocking CpG methylation in and around these sites. Binding sites for ETS-family factors, several of which are encoded by genes on HS21 (Fig. 5), also showed enrichment among DS-DM loci in some tissues (Do *et al.* 2017b). The observation that the “core set” of loci with strong DS-DM across multiple human tissues are significantly enriched in CTCF insulator binding sites

(Mendioroz *et al.* 2015; Do *et al.* 2017b) is also likely relevant. Since CTCF is not encoded on Hsa21, the connection between Ts21 and altered methylation at specific CTCF sites is presumably indirect, possibly mediated by changes in the amounts of other DNA binding proteins that are encoded on Hsa21 and associate with CTCF on chromatin, or through CTCF binding sites being hypersensitive to altered activity of methylation pathway enzymes and cofactors, several of which are encoded on Hsa21.

Further testing of the TF over-expression and binding site occupancy hypothesis will be relevant not only to DS but also for understanding the fundamental mechanisms of epigenetic patterning in mammalian cells. The occupancy of the binding sites of many proteins on DNA is methylation-sensitive can lead to protection of occupied sites from CpG methylation. These proteins includes not only CTCF (Williams and Flavell 2008; Feldmann *et al.* 2013; Ong and Corces 2014; Do *et al.* 2016), but also many others (Hu *et al.* 2013; Xu *et al.* 2015; Anvar *et al.* 2016), including ETS-family factors (Gaston and Fried 1995; Stephens and Poon 2016). Moreover, TF binding site occupancy even by methylation-insensitive factors can protect those sites from becoming CpG-methylated (reviewed in (Do *et al.* 2017a)). Thus, results from studying the role of Hsa21-encoded TFs and their binding site occupancies in shaping the patterns of DS-DM will likely help to consolidate our understanding of how DNA methylation patterns are established in normal development and how these patterns are altered not only in DS but also in human cancers, many of which are associated with recurrent chromosomal aneuploidies.

Does DS-DM affect gene expression and DS phenotypes?—The results from PBL and T lymphocytes indicate that DS-DM affects target genes encoding signaling proteins and transcription factors that are known or suspected to be necessary for lymphocyte and NK cell development and function (Kerkel *et al.* 2010; Bacalini *et al.* 2015; Mendioroz *et al.* 2015; Alves da Silva *et al.* 2016). Examples include *TMEM131*, encoding a trans-membrane protein that marks lymphocyte precursor cells, *SH3BP2*, coding for a signaling adaptor protein that has been best studied in B lymphocytes, *ZDHHC14*, which codes for a palmitoyl transferase that can regulate receptor tyrosine kinases, and *RUNX1*, which is crucial for hematopoietic stem/progenitor cell, NKT, and T cell development. DS-DM in these genes may play roles in the mild immunodeficiency and markedly increased autoimmune phenomena in DS. Likewise, a high percentage of the genes with DS-DM in neural cells, such as those encoding multiple protocadherin-family proteins, neuroligin-2, cytohesin-2, the signaling receptor amigo-3, the brsk-2/sad-a kinase, and others, have known or predicted roles in brain development or function (Mendioroz *et al.* 2015). Correlations of DS-DM with altered gene expression in human DS and in mouse models are under investigation, and our published (Kerkel *et al.* 2010) and unpublished data so far suggest that some, but not all, of the DS-DM loci have detectable effects on the expression of nearby genes. Such effects, when present, presumably occur via methylation-dependent alterations of the functions of the specific promoter, enhancer, or insulator elements that are affected by DS-DM. Even when a change in expression of nearby genes is not found, an interesting possibility is that DS-DM might be an adaptive cellular response to the perturbations of gene expression networks that are caused by the extra chromosome 21. According to this hypothesis, DS-DM at some loci might act as an epigenetic mechanism for keeping gene expression in the

normal range. These ideas can be actively tested in the future using genetic approaches in the mouse models of DS. These types of experiments will ultimately clarify whether the consequences of DS-DM at specific loci are adverse, neutral, or protective against the medically relevant DS-associated phenotypes.

Early epigenetic and biological aging in Down syndrome—Individuals with DS exhibit premature signs of aging, including wrinkled skin, gray hair, early menopause, hearing loss, declining immune function, increased autoimmune diseases with some of which can show age-dependence in humans and mice, and Alzheimer's disease (AD). Both human and mouse data have shown that *APP* triplication is required for early-onset AD in DS (Prasher *et al.* 1998; Jiang *et al.* 2010), so early onset of AD in this syndrome might be largely a mimic or “phenocopy” of early aging, not actually due to it. Alternatively, increased *APP* gene dosage might be acting in concert with bona fide early aging to accelerate the onset of this disease (Devenny *et al.* 2005; Head *et al.* 2012). According to this hypothesis, the age-at-onset of AD in DS is dictated by multiple modifier genes, including some that are not located on Hsa21 but have biological roles in AD pathology and are genetically polymorphic in human populations (Schupf *et al.* 2015; Lee *et al.* 2017; Schupf *et al.* 2018), and others, aside from *APP*, that reside on Hsa21 and might affect the aging process *per se*. Moreover, early aging of other organs might rather be due to the triplications of other Hsa21 gene orthologs, some of which might directly influence the aging process.

Using a methodology pioneered by Steve Horvath (Horvath 2013), DNA methylation patterns can be utilized as an “epigenetic clock” that correlates with chronological age in humans and mice (Thompson *et al.* 2018). The basic approach is to form a weighted average of fractional methylation levels of a set of “clock CpGs” (identified by Horvath and colleagues using an elastic net logistic regression model applied to multiple public methylation datasets that included the ages of each sample), which is then transformed to DNA methylation age of a given newly analyzed sample using a calibration function. Differences between methylation and chronological age can be defined as the residual that results from regressing DNA methylation age on chronological age.

Both Horvath and his collaborators (Horvath *et al.* 2015; Franceschi *et al.* 2019) and our group (Mendioroz *et al.* 2015) have examined epigenetic aging in human DS, using separate sample sets. Horvath *et al.* concluded that there is accelerated aging of methylation patterns in blood and brains of individuals with DS (Horvath *et al.* 2015). Our analysis of the same data, plus our own 450K data from brains and T cells, showed faster aging of CpG methylation patterns during fetal and possibly early post-natal development in DS, leading to a higher initial “set point” of epigenetic age, but no evidence of accelerated aging of these patterns during adult life (Mendioroz *et al.* 2015). In making this interpretation, the salient features of the X-Y plots of methylation age vs chronological age are the slope and the Y-intercept. As shown for our 450K methylation data from human DS vs sex-and age-matched control T cell samples in Figure 4C, the slope of the DS fitted line (the rate of aging) does not differ between DS and controls. Rather, the difference in DNA methylation age at each chronological age is fully accounted for by the higher Y-intercept of the DS fitted line, which indicates that the “set point” of epigenetic aging is established early in life. We have even detected this advancement in epigenetic age using our series of DS and control fetal

cerebrum samples (Mendioroz *et al.* 2015). Thus, we can conclude that the rate of epigenetic aging is not altered in adult DS cells and tissues, but rather the advancement of epigenetic age, as determined from the Horvath DNA methylation clock CpGs, is fully established early in development. To emphasize this important distinction, we refer to “early epigenetic aging”, but not “accelerated epigenetic aging” in DS. While additional work is needed, both in human samples and in mouse models, we believe that this conclusion regarding the early establishment of the advancement in epigenetic age in DS, and lack of acceleration of epigenetic aging in adult life, will need to be considered when investigating the mechanisms of biological aging in this syndrome.

Lastly and importantly, while the evidence discussed above suggests that both epigenetic aging and DS-DM are established early in development, and therefore might share some underlying mechanisms, the phenomenon of epigenetic aging in DS, as measured using the panel of several hundred Horvath CpGs, is clearly distinct from the phenomenon of strong DS-DM, which involves different sets of several thousand CpGs. While highly predictive of chronological age in aggregate, the changes in fractional methylation of individual CpGs during aging are typically small, and the early epigenetic aging detected in DS reflects small magnitude changes in fractional methylation of each CpG in the Horvath panel. In contrast, the strong DS-DM loci identified in case-control comparisons of brain cells and tissues, and whole blood and T-lymphocytes, are not accounted for by age effects: in our 450K methylation data from these tissues and cell types, fewer than 5 percent of DS-DM CpGs show evidence of age-dependent methylation (Mendioroz *et al.* 2015).

Challenges for future work and potential therapeutic implications—Genetic dissections based on partial human Ts21 and animal models have facilitated and will continue to facilitate identifications of the critical genomic regions and dosage-sensitive causative genes associated with clinical phenotypes in DS. Understanding how these genes contribute to the phenotypes is helping us unravel the pathogenic mechanisms, which in turn offers opportunities for developing therapeutic interventions. Preclinical experimental studies on *Dyrk1a* and the clinical trials associated with the inhibitors of the protein product encoded by this Hsa21 gene are a fruitful example (De la Torre *et al.* 2014; de la Torre *et al.* 2016), motivating further efforts in this important area.

As we have proposed previously (Do *et al.* 2017b), given the potential phenotypic impacts of DS-DM it is worth taking epigenetic pathways into account for future therapies. Dietary manipulations that are non-toxic and seem to be safe in the post-natal period can affect DNA methylation patterns. The classical example is methyl-donor supplementation with folic acid, betaine, and vitamin B12. Such diets, which can help to maintain methylation levels genome-wide, have been administered to individuals with DS in a few controlled studies, with variable but partly promising effects on measures of overall health ((Blehaut *et al.* 2010); references therein). Similarly, Jill James and colleagues showed that the altered balance of metabolites in SAM-dependent methylation pathways in cells with Ts21 could be ameliorated by addition of methionine, folinic acid, methyl-B12, thymidine, or dimethylglycine to the cells in culture (Pogribna *et al.* 2001). Methyl donor-rich diets would not reduce the methylation of the DS-DM loci that are *hypermethylated* (e.g. most DS-DM loci in brain cells) but might prevent losses of methylation at DS-DM loci that are

hypomethylated, of which there are good number in whole blood leukocytes and T cells. Whether such changes would be beneficial for immune function in DS depends on whether DS-DM in blood cells is an adverse or beneficial (compensatory) phenomenon – which is still an unanswered question. More generally, the identification of specific DS-DM target genes that play roles in DS-associated phenotypes such as immune dysregulation and intellectual disability may provide clues to future targeted therapies, both in people with DS and in the wider population.

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REFERENCES

- Abuhatzira L, Shamir A, Schones DE, Schaffer AA and Bustin M. (2011) The chromatin-binding protein HMGN1 regulates the expression of methyl CpG-binding protein 2 (MECP2) and affects the behavior of mice. *J Biol Chem* 286: 42051–42062 [PubMed: 22009741]
- Adams DJ, Biggs PJ, Cox T, Davies R, van der Weyden L, Jonkers J, Smith J, Plumb B, Taylor R, Nishijima I, Yu Y, Rogers J. and Bradley A. (2004) Mutagenic insertion and chromosome engineering resource (MICER). *Nat Genet* 36: 867–871 [PubMed: 15235602]
- Alves da Silva AF, Machado FB, Pavarino EC, Biselli-Perico JM, Zampieri BL, da Silva Francisco R Junior, Mozer Rodrigues PT, Terra Machado D, Santos-Reboucas CB, Gomes Fernandes M, Chuva de Sousa Lopes SM, Lopes Rios AF. and Medina-Acosta E. (2016) Trisomy 21 Alters DNA Methylation in Parent-of-Origin-Dependent and -Independent Manners. *PLoS One* 11: e0154108
- Anvar Z, Cammisa M, Riso V, Baglivo I, Kukreja H, Sparago A, Girardot M, Lad S, De Feis I, Cerrato F, Angelini C, Feil R, Pedone PV, Grimaldi G. and Riccio A. (2016) ZFP57 recognizes multiple and closely spaced sequence motif variants to maintain repressive epigenetic marks in mouse embryonic stem cells. *Nucleic acids research* 44: 1118–1132 [PubMed: 26481358]
- Bacalini MG, Gentilini D, Boattini A, Giampieri E, Pirazzini C, Giuliani C, Fontanesi E, Scurti M, Remondini D, Capri M, Cocchi G, Ghezzi A, Del Rio A, Luiselli D, Vitale G, Mari D, Castellani G, Fraga M, Di Blasio AM, Salvioli S, Franceschi C. and Garagnani P. (2015) Identification of a DNA methylation signature in blood cells from persons with Down Syndrome. *Aging (Albany NY)* 7: 82–96 [PubMed: 25701644]
- Barlow GM, Micales B, Lyons GE and Korenberg JR (2001) Down syndrome cell adhesion molecule is conserved in mouse and highly expressed in the adult mouse brain. *Cytogenet Cell Genet* 94: 155–162 [PubMed: 11856873]
- Belichenko NP, Belichenko PV, Kleschevnikov AM, Salehi A, Reeves RH and Mobley WC (2009) The “Down syndrome critical region” is sufficient in the mouse model to confer behavioral, neurophysiological, and synaptic phenotypes characteristic of Down syndrome. *J Neurosci* 29: 5938–5948 [PubMed: 19420260]
- Birling MC, Schaeffer L, Andre P, Lindner L, Marechal D, Ayadi A, Sorg T, Pavlovic G. and Herault Y. (2017) Efficient and rapid generation of large genomic variants in rats and mice using CRISMER. *Sci Rep* 7: 43331 [PubMed: 28266534]
- Blehaut H, Mircher C, Ravel A, Conte M, de Portzamparc V, Poret G, de Kermadec FH, Rethore MO and Sturtz FG (2010) Effect of leucovorin (folinic acid) on the developmental quotient of children with Down's syndrome (trisomy 21) and influence of thyroid status. *PLoS One* 5: e8394 [PubMed: 20084109]
- Bons N, Rieger F, Prudhomme D, Fisher A. and Krause KH (2006) *Microcebus murinus*: a useful primate model for human cerebral aging and Alzheimer's disease? *Genes Brain Behav* 5: 120–130 [PubMed: 16507003]

- Boroviak K, Doe B, Banerjee R, Yang F. and Bradley A. (2016) Chromosome engineering in zygotes with CRISPR/Cas9. *Genesis* 54: 78–85 [PubMed: 26742453]
- Boroviak K, Fu B, Yang F, Doe B. and Bradley A. (2017) Revealing hidden complexities of genomic rearrangements generated with Cas9. *Sci Rep* 7: 12867 [PubMed: 28993641]
- Bourc'his D, Xu GL, Lin CS, Bollman B. and Bestor TH (2001) Dnmt3L and the establishment of maternal genomic imprints. *Science* 294: 2536–2539 [PubMed: 11719692]
- Condon DE, Tran PV, Lien YC, Schug J, Georgieff MK, Simmons RA and Won KJ (2018) Defiant: (DMRs: easy, fast, identification and ANnotation) identifies differentially Methylated regions from iron-deficient rat hippocampus. *BMC Bioinformatics* 19: 31 [PubMed: 29402210]
- Costa V, Angelini C, D'Apice L, Mutarelli M, Casamassimi A, Sommese L, Gallo MA, Aprile M, Esposito R, Leone L, Donizetti A, Crispi S, Rienzo M, Sarubbi B, Calabro R, Picardi M, Salvatore P, Infante T, De Berardinis P, Napoli C. and Ciccociola A. (2011) Massive-scale RNA-Seq analysis of non ribosomal transcriptome in human trisomy 21. *PLoS One* 6: e18493
- Davisson MT, Schmidt C. and Akeson EC (1990) Segmental trisomy of murine chromosome 16: a new model system for studying Down syndrome. *Prog Clin Biol Res* 360: 263–280 [PubMed: 2147289]
- de la Torre R, de Sola S, Hernandez G, Farre M, Pujol J, Rodriguez J, Espadaler JM, Langohr K, Cuenca-Royo A, Principe A, Xicota L, Janel N, Catuara-Solarz S, Sanchez-Benavides G, Blehaut H, Duenas-Espin I, Del Hoyo L, Benejam B, Blanco-Hinojo L, Videla S, Fito M, Delabar JM, Dierssen M. and group T. s. (2016) Safety and efficacy of cognitive training plus epigallocatechin-3-gallate in young adults with Down's syndrome (TESDAD): a double-blind, randomised, placebo-controlled, phase 2 trial. *Lancet Neurol* 15: 801–810 [PubMed: 27302362]
- De la Torre R, De Sola S, Pons M, Duchon A, de Lagran MM, Farre M, Fito M, Benejam B, Langohr K, Rodriguez J, Pujadas M, Bizot JC, Cuenca A, Janel N, Catuara S, Covas MI, Blehaut H, Herault Y, Delabar JM and Dierssen M. (2014) Epigallocatechin-3-gallate, a DYRK1A inhibitor, rescues cognitive deficits in Down syndrome mouse models and in humans. *Mol Nutr Food Res* 58: 278–288 [PubMed: 24039182]
- Dekker AD, De Deyn PP and Rots MG (2014) Epigenetics: the neglected key to minimize learning and memory deficits in Down syndrome. *Neurosci Biobehav Rev* 45: 72–84 [PubMed: 24858130]
- Devenny DA, Wegiel J, Schupf N, Jenkins E, Zigman W, Krinsky-McHale SJ and Silverman WP (2005) Dementia of the Alzheimer's type and accelerated aging in Down syndrome. *Sci Aging Knowledge Environ* 2005: dn1
- Do C, Lang CF, Lin J, Darbary H, Krupska I, Gaba A, Petukhova L, Vonsattel JP, Gallagher MP, Goland RS, Clynes RA, Dwork A, Kral JG, Monk C, Christiano AM and Tycko B. (2016) Mechanisms and Disease Associations of Haplotype-Dependent Allele-Specific DNA Methylation. *Am J Hum Genet* 98: 934–955 [PubMed: 27153397]
- Do C, Shearer A, Suzuki M, Terry MB, Gelernter J, Greally JM and Tycko B. (2017a) Genetic-epigenetic interactions in cis: a major focus in the post-GWAS era. *Genome Biol* 18: 120 [PubMed: 28629478]
- Do C, Xing Z, Yu YE and Tycko B. (2017b) Trans-acting epigenetic effects of chromosomal aneuploidies: lessons from Down syndrome and mouse models. *Epigenomics* 9: 189–207 [PubMed: 27911079]
- Down JL (1887). On some of the mental affections of childhood and youth: being the Lettsomian lectures delivered before the Medical Society of London in 1887: together with other papers. London, J & A Churchill.
- Eckmann-Scholz C, Bens S, Kolarova J, Schneppenheim S, Caliebe A, Heidemann S, von Kaisenberg C, Kautza M, Jonat W, Siebert R. and Ammerpohl O. (2012) DNA-methylation profiling of fetal tissues reveals marked epigenetic differences between chorionic and amniotic samples. *PLoS One* 7: e39014
- Egeo A, Mazzocco M, Sotgia F, Arrigo P, Oliva R, Bergonon S, Nizetic D, Rasore-Quartino A. and Scartezzini P. (1998) Identification and characterization of a new human cDNA from chromosome 21q22.3 encoding a basic nuclear protein. *Hum Genet* 102: 289–293 [PubMed: 9544840]

- El Hajj N, Dittrich M, Bock J, Kraus TF, Nanda I, Muller T, Seidmann L, Tralau T, Galetzka D, Schneider E. and Haaf T. (2016) Epigenetic dysregulation in the developing Down syndrome cortex. *Epigenetics* 11: 563–578 [PubMed: 27245352]
- Feldmann A, Ivanek R, Murr R, Gaidatzis D, Burger L. and Schubeler D. (2013) Transcription factor occupancy can mediate active turnover of DNA methylation at regulatory regions. *PLoS Genet* 9: e1003994
- Fernandez-Martinez J, Vela EM, Tora-Ponsioen M, Ocana OH, Nieto MA and Galceran J. (2009) Attenuation of Notch signalling by the Down-syndrome-associated kinase DYRK1A. *J Cell Sci* 122: 1574–1583 [PubMed: 19383720]
- Fotaki V, Dierssen M, Alcantara S, Martinez S, Marti E, Casas C, Visa J, Soriano E, Estivill X. and Arbones ML (2002) Dyrk1A haploinsufficiency affects viability and causes developmental delay and abnormal brain morphology in mice. *Mol Cell Biol* 22: 6636–6647 [PubMed: 12192061]
- Franceschi C, Garagnani P, Gensous N, Bacalini MG, Conte M. and Salvioli S. (2019) Accelerated bio-cognitive aging in Down syndrome: State of the art and possible deceleration strategies. *Aging Cell*: e12903
- Gaston K. and Fried M. (1995) CpG methylation has differential effects on the binding of YY1 and ETS proteins to the bi-directional promoter of the Surf-1 and Surf-2 genes. *Nucleic Acids Res* 23: 901–909 [PubMed: 7731802]
- Gu B, Posfai E. and Rossant J. (2018) Efficient generation of targeted large insertions by microinjection into two-cell-stage mouse embryos. *Nat Biotechnol* 36: 632–637 [PubMed: 29889212]
- Hammerle B, Ulin E, Guimera J, Becker W, Guillemot F. and Tejedor FJ (2011) Transient expression of Mnb/Dyrk1a couples cell cycle exit and differentiation of neuronal precursors by inducing p27KIP1 expression and suppressing NOTCH signaling. *Development* 138: 2543–2554 [PubMed: 21610031]
- Hatt L, Aagaard MM, Bach C, Graakjaer J, Sommer S, Agerholm IE, Kolvraa S. and Bojesen A. (2016) Microarray-Based Analysis of Methylation of 1st Trimester Trisomic Placentas from Down Syndrome, Edwards Syndrome and Patau Syndrome. *PLoS One* 11: e0160319
- Head E, Silverman W, Patterson D. and Lott IT (2012) Aging and down syndrome. *Curr Gerontol Geriatr Res* 2012: 412536
- Henneman P, Bouman A, Mul A, Knecht L, van der Kevie-Kersemaekers AM, Zwaveling-Soonawala N, Meijers-Heijboer HEJ, van Trotsenburg ASP and Mannens MM (2018) Widespread domain-like perturbations of DNA methylation in whole blood of Down syndrome neonates. *PLoS One* 13: e0194938
- Herauld Y, Delabar JM, Fisher EMC, Tybulewicz VLJ, Yu E. and Brault V. (2017) Rodent models in Down syndrome research: impact and future opportunities. *Dis Model Mech* 10: 1165–1186 [PubMed: 28993310]
- Horvath S. (2013) DNA methylation age of human tissues and cell types. *Genome Biol* 14: R115 [PubMed: 24138928]
- Horvath S, Garagnani P, Bacalini MG, Pirazzini C, Salvioli S, Gentilini D, Di Blasio AM, Giuliani C, Tung S, Vinters HV and Franceschi C. (2015) Accelerated epigenetic aging in Down syndrome. *Aging cell* 14: 491–495 [PubMed: 25678027]
- Hu S, Wan J, Su Y, Song Q, Zeng Y, Nguyen HN, Shin J, Cox E, Rho HS, Woodard C, Xia S, Liu S, Lyu H, Ming GL, Wade H, Song H, Qian J. and Zhu H. (2013) DNA methylation presents distinct binding sites for human transcription factors. *Elife* 2: e00726 [PubMed: 24015356]
- Ilbery PL, Lee CW and Winn SM (1961) Incomplete trisomy in a mongoloid child exhibiting minimal stigmata. *Med J Aust* 48(2): 182–184 [PubMed: 13717495]
- Jennings CG, Landman R, Zhou Y, Sharma J, Hyman J, Movshon JA, Qiu Z, Roberts AC, Roe AW, Wang X, Zhou H, Wang L, Zhang F, Desimone R. and Feng G. (2016) Opportunities and challenges in modeling human brain disorders in transgenic primates. *Nat Neurosci* 19: 1123–1130 [PubMed: 27571191]
- Jiang X, Liu C, Yu T, Zhang L, Meng K, Xing Z, Belichenko PV, Kleschevnikov AM, Pao A, Peresie J, Wie S, Mobley WC and Yu YE (2015) Genetic dissection of the Down syndrome critical region. *Hum Mol Genet* 24: 6540–6551 [PubMed: 26374847]

- Jiang Y, Mullaney KA, Peterhoff CM, Che S, Schmidt SD, Boyer-Boiteau A, Ginsberg SD, Cataldo AM, Mathews PM and Nixon RA (2010) Alzheimer's-related endosome dysfunction in Down syndrome is Abeta-independent but requires APP and is reversed by BACE-1 inhibition. *Proc Natl Acad Sci U S A* 107: 1630–1635 [PubMed: 20080541]
- Jin S, Lee YK, Lim YC, Zheng Z, Lin XM, Ng DP, Holbrook JD, Law HY, Kwek KY, Yeo GS and Ding C. (2013) Global DNA hypermethylation in down syndrome placenta. *PLoS Genet* 9: e1003515
- Jones MJ, Farre P, McEwen LM, Macisaac JL, Watt K, Neumann SM, Emberly E, Cynader MS, Virji-Babul N. and Kobor MS (2013) Distinct DNA methylation patterns of cognitive impairment and trisomy 21 in Down syndrome. *BMC Med Genomics* 6: 58 [PubMed: 24373378]
- Kahlem P, Sultan M, Herwig R, Steinfath M, Balzereit D, Eppens B, Saran NG, Pletcher MT, South ST, Stetten G, Lehrach H, Reeves RH and Yaspo ML (2004) Transcript level alterations reflect gene dosage effects across multiple tissues in a mouse model of Down syndrome. *Genome Res* 14: 1258–1267 [PubMed: 15231742]
- Kelly PA and Rahmani Z. (2005) DYRK1A enhances the mitogen-activated protein kinase cascade in PC12 cells by forming a complex with Ras, B-Raf, and MEK1. *Mol Biol Cell* 16: 3562–3573 [PubMed: 15917294]
- Kerkel K, Schupf N, Hatta K, Pang D, Salas M, Kratz A, Minden M, Murty V, Zigman WB, Mayeux RP, Jenkins EC, Torkamani A, Schork NJ, Silverman W, Croy BA and Tycko B. (2010) Altered DNA methylation in leukocytes with trisomy 21. *PLoS genetics* 6: e1001212
- Kim IS, Lee M, Park KC, Jeon Y, Park JH, Hwang EJ, Jeon TI, Ko S, Lee H, Baek SH and Kim KI (2012) Roles of Mis18alpha in epigenetic regulation of centromeric chromatin and CENP-A loading. *Mol Cell* 46: 260–273 [PubMed: 22516971]
- Kleschevnikov AM, Yu J, Kim J, Lysenko LV, Zeng Z, Yu YE and Mobley WC (2017) Evidence that increased *Kcnj6* gene dose is necessary for deficits in behavior and dentate gyrus synaptic plasticity in the Ts65Dn mouse model of Down syndrome. *Neurobiol Dis* 103: 1–10 [PubMed: 28342823]
- Korbel JO, Tirosh-Wagner T, Urban AE, Chen XN, Kasowski M, Dai L, Grubert F, Erdman C, Gao MC, Lange K, Sobel EM, Barlow GM, Aylsworth AS, Carpenter NJ, Clark RD, Cohen MY, Doran E, Falik-Zaccai T, Lewin SO, Lott IT, McGillivray BC, Moeschler JB, Pettenati MJ, Puschel SM, Rao KW, Shaffer LG, Shohat M, Van Riper AJ, Warburton D, Weissman S, Gerstein MB, Snyder M. and Korenberg JR (2009) The genetic architecture of Down syndrome phenotypes revealed by high-resolution analysis of human segmental trisomies. *Proc Natl Acad Sci U S A* 106: 12031–12036 [PubMed: 19597142]
- Kraft K, Geuer S, Will AJ, Chan WL, Paliou C, Borschiwer M, Harabula I, Wittler L, Franke M, Ibrahim DM, Kragestein BK, Spielmann M, Mundlos S, Lupianez DG and Andrey G. (2015) Deletions, Inversions, Duplications: Engineering of Structural Variants using CRISPR/Cas in Mice. *Cell Rep* 10: 833–839 [PubMed: 25660031]
- Lagutina IV, Valentine V, Picchione F, Harwood F, Valentine MB, Villarejo-Balcells B, Carvajal JJ and Grosveld GC (2015) Modeling of the human alveolar rhabdomyosarcoma Pax3-Foxo1 chromosome translocation in mouse myoblasts using CRISPR-Cas9 nuclease. *PLoS Genet* 11: e1004951
- Languille S, Blanc S, Blin O, Canale CI, Dal-Pan A, Devau G, Dhenain M, Dorieux O, Epelbaum J, Gomez D, Hardy I, Henry PY, Irving EA, Marchal J, Mestre-Frances N, Perret M, Picq JL, Pifferi F, Rahman A, Schenker E, Terrien J, Thery M, Verdier JM and Aujard F. (2012) The grey mouse lemur: a non-human primate model for ageing studies. *Ageing Res Rev* 11: 150–162 [PubMed: 21802530]
- Lee JH, Lee AJ, Dang LH, Pang D, Kisselev S, Krinsky-McHale SJ, Zigman WB, Luchsinger JA, Silverman W, Tycko B, Clark LN and Schupf N. (2017) Candidate gene analysis for Alzheimer's disease in adults with Down syndrome. *Neurobiol Aging* 56: 150–158 [PubMed: 28554490]
- LeJeune J, Gautier M. and Turpin R. (1959) Etudes des chromosomes somatiques de neuf enfants mongoliens. *Comptes Rendus de l'Académie des Sciences* 248: 1721–1722
- Letourneau A, Santoni FA, Bonilla X, Sailani MR, Gonzalez D, Kind J, Chevalier C, Thurman R, Sandstrom RS, Hibaoui Y, Garieri M, Popadin K, Falconnet E, Gagnebin M, Gehrig C, Vannier A, Guipponi M, Farinelli L, Robyr D, Migliaiavacca E, Borel C, Deutsch S, Feki A,

- Stamatoyannopoulos JA, Herault Y, van Steensel B, Guigo R. and Antonarakis SE (2014) Domains of genome-wide gene expression dysregulation in Down's syndrome. *Nature* 508: 345–350 [PubMed: 24740065]
- Li CM, Guo M, Salas M, Schupf N, Silverman W, Zigman WB, Husain S, Warburton D, Thaker H. and Tycko B. (2006) Cell type-specific over-expression of chromosome 21 genes in fibroblasts and fetal hearts with trisomy 21. *BMC Med Genet* 7: 24 [PubMed: 16539728]
- Lim JH, Kim SY, Han JY, Kim MY, Park SY and Ryu HM (2016) Comprehensive investigation of DNA methylation and gene expression in trisomy 21 placenta. *Placenta* 42: 17–24 [PubMed: 27238709]
- Ling KH, Hewitt CA, Tan KL, Cheah PS, Vidyadaran S, Lai MI, Lee HC, Simpson K, Hyde L, Pritchard MA, Smyth GK, Thomas T. and Scott HS (2014) Functional transcriptome analysis of the postnatal brain of the Ts1Cje mouse model for Down syndrome reveals global disruption of interferon-related molecular networks. *BMC Genomics* 15: 624 [PubMed: 25052193]
- Lyle R, Bena F, Gagos S, Gehrig C, Lopez G, Schinzel A, Lespinasse J, Bottani A, Dahoun S, Taine L, Doco-Fenzy M, Cornillet-Lefebvre P, Pelet A, Lyonnet S, Toutain A, Colleaux L, Horst J, Kennerknecht I, Wakamatsu N, Descartes M, Franklin JC, Florentin-Arar L, Kitsiou S, Ait Yahya-Graison E, Costantine M, Sinet PM, Delabar JM and Antonarakis SE (2009) Genotype-phenotype correlations in Down syndrome identified by array CGH in 30 cases of partial trisomy and partial monosomy chromosome 21. *Eur J Hum Genet* 17: 454–466 [PubMed: 19002211]
- Lyle R, Gehrig C, Neergaard-Henrichsen C, Deutsch S. and Antonarakis SE (2004) Gene expression from the aneuploid chromosome in a trisomy mouse model of Down syndrome. *Genome Res* 14: 1268–1274 [PubMed: 15231743]
- Mao R, Zielke CL, Zielke HR and Pevsner J. (2003) Global up-regulation of chromosome 21 gene expression in the developing Down syndrome brain. *Genomics* 81: 457–467 [PubMed: 12706104]
- Mark MD and Herlitze S. (2000) G-protein mediated gating of inward-rectifier K⁺ channels. *Eur J Biochem* 267: 5830–5836 [PubMed: 10998041]
- Matevossian A. and Akbarian S. (2008) Neuronal nuclei isolation from human postmortem brain tissue. *Journal of visualized experiments : JoVE*
- Mendioroz M, Do C, Jiang X, Liu C, Darbary HK, Lang CF, Lin J, Thomas A, Abu-Amro S, Stanier P, Temkin A, Yale A, Liu MM, Li Y, Salas M, Kerkel K, Capone G, Silverman W, Yu YE, Moore G, Wegiel J. and Tycko B. (2015) Trans effects of chromosome aneuploidies on DNA methylation patterns in human Down syndrome and mouse models. *Genome biology* 16: 263 [PubMed: 26607552]
- Nadal M, Vigo CG, Melaragno MI, Andrade JA, Alonso LG, Brunoni D, Pritchard M. and Estivill X. (2001) Clinical and cytogenetic characterisation of a patient with Down syndrome resulting from a 21q22.1-->qter duplication. *J Med Genet* 38: 73–76 [PubMed: 11334012]
- Niu Y, Shen B, Cui Y, Chen Y, Wang J, Wang L, Kang Y, Zhao X, Si W, Li W, Xiang AP, Zhou J, Guo X, Bi Y, Si C, Hu B, Dong G, Wang H, Zhou Z, Li T, Tan T, Pu X, Wang F, Ji S, Zhou Q, Huang X, Ji W. and Sha J. (2014) Generation of gene-modified cynomolgus monkey via Cas9/RNA-mediated gene targeting in one-cell embryos. *Cell* 156: 836–843 [PubMed: 24486104]
- O'Doherty A, Ruf S, Mulligan C, Hildreth V, Errington ML, Cooke S, Sesay A, Modino S, Vanes L, Hernandez D, Linehan JM, Sharpe PT, Brandner S, Bliss TV, Henderson DJ, Nizetic D, Tybulewicz VL and Fisher EM (2005) An aneuploid mouse strain carrying human chromosome 21 with Down syndrome phenotypes. *Science* 309: 2033–2037 [PubMed: 16179473]
- Ong CT and Corces VG (2014) CTCF: an architectural protein bridging genome topology and function. *Nat Rev Genet* 15: 234–246 [PubMed: 24614316]
- Ooi SK, Qiu C, Bernstein E, Li K, Jia D, Yang Z, Erdjument-Bromage H, Tempst P, Lin SP, Allis CD, Cheng X. and Bestor TH (2007) DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. *Nature* 448: 714–717 [PubMed: 17687327]
- Pelleri MC, Cicchini E, Locatelli C, Vitale L, Caracausi M, Piovesan A, Rocca A, Poletti G, Seri M, Strippoli P. and Cocchi G. (2016) Systematic reanalysis of partial trisomy 21 cases with or without Down syndrome suggests a small region on 21q22.13 as critical to the phenotype. *Hum Mol Genet* 25: 2525–2538 [PubMed: 27106104]

- Pogribna M, Melnyk S, Pogribny I, Chango A, Yi P. and James SJ (2001) Homocysteine metabolism in children with Down syndrome: in vitro modulation. *Am J Hum Genet* 69: 88–95 [PubMed: 11391481]
- Prandini P, Deutsch S, Lyle R, Gagnebin M, Delucinge Vivier C, Delorenzi M, Gehrig C, Descombes P, Sherman S, Dagna Bricarelli F, Baldo C, Novelli A, Dallapiccola B. and Antonarakis SE (2007) Natural gene-expression variation in Down syndrome modulates the outcome of gene-dosage imbalance. *Am J Hum Genet* 81: 252–263 [PubMed: 17668376]
- Prasher VP, Farrer MJ, Kessling AM, Fisher EM, West RJ, Barber PC and Butler AC (1998) Molecular mapping of Alzheimer-type dementia in Down's syndrome. *Ann Neurol* 43: 380–383 [PubMed: 9506555]
- Renelt M, von Bohlen und Halbach V. and von Bohlen und Halbach O. (2014) Distribution of PCP4 protein in the forebrain of adult mice. *Acta Histochem* 116: 1056–1061 [PubMed: 24954028]
- Sailani MR, Santoni FA, Letourneau A, Borel C, Makrythanasis P, Hibaoui Y, Popadin K, Bonilla X, Guipponi M, Gehrig C, Vannier A, Carre-Pigeon F, Feki A, Nizetic D. and Antonarakis SE (2015) DNA-Methylation Patterns in Trisomy 21 Using Cells from Monozygotic Twins. *PLoS One* 10: e0135555
- Saran NG, Pletcher MT, Natale JE, Cheng Y. and Reeves RH (2003) Global disruption of the cerebellar transcriptome in a Down syndrome mouse model. *Hum Mol Genet* 12: 2013–2019 [PubMed: 12913072]
- Sasaki E, Suemizu H, Shimada A, Hanazawa K, Oiwa R, Kamioka M, Tomioka I, Sotomaru Y, Hirakawa R, Eto T, Shiozawa S, Maeda T, Ito M, Ito R, Kito C, Yagihashi C, Kawai K, Miyoshi H, Tanioka Y, Tamaoki N, Habu S, Okano H. and Nomura T. (2009) Generation of transgenic non-human primates with germline transmission. *Nature* 459: 523–527 [PubMed: 19478777]
- Schupf N, Lee A, Park N, Dang LH, Pang D, Yale A, Oh DK, Krinsky-McHale SJ, Jenkins EC, Luchsinger JA, Zigman WB, Silverman W, Tycko B, Kisselev S, Clark L. and Lee JH (2015) Candidate genes for Alzheimer's disease are associated with individual differences in plasma levels of beta amyloid peptides in adults with Down syndrome. *Neurobiol Aging* 36: 2907 e2901–2910
- Schupf N, Lee JH, Pang D, Zigman WB, Tycko B, Krinsky-McHale S. and Silverman W. (2018) Epidemiology of estrogen and dementia in women with Down syndrome. *Free Radic Biol Med* 114: 62–68 [PubMed: 28843780]
- Song HJ, Park J, Seo SR, Kim J, Paik SR and Chung KC (2008) Down syndrome critical region 2 protein inhibits the transcriptional activity of peroxisome proliferator-activated receptor beta in HEK293 cells. *Biochem Biophys Res Commun* 376: 478–482 [PubMed: 18793612]
- Stephens DC and Poon GM (2016) Differential sensitivity to methylated DNA by ETS-family transcription factors is intrinsically encoded in their DNA-binding domains. *Nucleic Acids Res* 44: 8671–8681 [PubMed: 27270080]
- Sullivan KD, Lewis HC, Hill AA, Pandey A, Jackson LP, Cabral JM, Smith KP, Liggett LA, Gomez EB, Galbraith MD, DeGregori J. and Espinosa JM (2016) Trisomy 21 consistently activates the interferon response. *Elife* 5
- Tardif SD, Mansfield KG, Ratnam R, Ross CN and Ziegler TE (2011) The marmoset as a model of aging and age-related diseases. *ILAR J* 52: 54–65 [PubMed: 21411858]
- Thompson MJ, Chwialkowska K, Rubbi L, Lusis AJ, Davis RC, Srivastava A, Korstanje R, Churchill GA, Horvath S. and Pellegrini M. (2018) A multi-tissue full lifespan epigenetic clock for mice. *Aging (Albany NY)* 10: 2832–2854 [PubMed: 30348905]
- Vacik T, Ort M, Gregorova S, Strnad P, Blatny R, Conte N, Bradley A, Bures J. and Forejt J. (2005) Segmental trisomy of chromosome 17: a mouse model of human aneuploidy syndromes. *Proc Natl Acad Sci U S A* 102: 4500–4505 [PubMed: 15755806]
- Vidal-Taboada JM, Lu A, Pique M, Pons G, Gil J. and Oliva R. (2000) Down syndrome critical region gene 2: expression during mouse development and in human cell lines indicates a function related to cell proliferation. *Biochem Biophys Res Commun* 272: 156–163 [PubMed: 10872820]
- Wang H, Yang H, Shivalila CS, Dawlaty MM, Cheng AW, Zhang F. and Jaenisch R. (2013) One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. *Cell* 153: 910–918 [PubMed: 23643243]

- Watson-Scales S, Kalmar B, Lana-Elola E, Gibbins D, La Russa F, Wiseman F, Williamson M, Saccon R, Slender A, Olerinyova A, Mahmood R, Nye E, Cater H, Wells S, Yu YE, Bennett DLH, Greensmith L, Fisher EMC and Tybulewicz VLJ (2018) Analysis of motor dysfunction in Down Syndrome reveals motor neuron degeneration. *PLoS Genet* 14: e1007383
- Williams A. and Flavell RA (2008) The role of CTCF in regulating nuclear organization. *J Exp Med* 205: 747–750 [PubMed: 18347103]
- Wolvetang EJ, Wilson TJ, Sanij E, Busciglio J, Hatzistavrou T, Seth A, Hertzog PJ and Kola I. (2003a) ETS2 overexpression in transgenic models and in Down syndrome predisposes to apoptosis via the p53 pathway. *Human Molecular Genetics* 12: 247–255 [PubMed: 12554679]
- Wolvetang EW, Bradfield OM, Tymms M, Zavarsek S, Hatzistavrou T, Kola I. and Hertzog PJ (2003b) The chromosome 21 transcription factor ETS2 transactivates the beta-APP promoter: implications for Down syndrome. *Biochim Biophys Acta* 1628: 105–110 [PubMed: 12890557]
- Xing Z, Li Y, Pao A, Bennett AS, Tycko B, Mobley WC and Yu YE (2016) Mouse-based genetic modeling and analysis of Down syndrome. *Br Med Bull* 120: 111–122 [PubMed: 27789459]
- Xing Z, Li Y, Pao A, Kaas G. and Yu YE (2018). CRISPR/Cas9-Facilitated Chromosome Engineering to Model Human Chromosomal Alterations. *Advances in Research on Down Syndrome*. Dey, S., IntechOpen: 209.
- Xu T, Li B, Zhao M, Szulwach KE, Street RC, Lin L, Yao B, Zhang F, Jin P, Wu H. and Qin ZS (2015) Base-resolution methylation patterns accurately predict transcription factor bindings in vivo. *Nucleic acids research* 43: 2757–2766 [PubMed: 25722376]
- Yamada M, Inanobe A. and Kurachi Y. (1998) G protein regulation of potassium ion channels. *Pharmacol Rev* 50: 723–760 [PubMed: 9860808]
- Yamakawa K, Huot YK, Haendelt MA, Hubert R, Chen XN, Lyons GE and Korenberg JR (1998) DSCAM: a novel member of the immunoglobulin superfamily maps in a Down syndrome region and is involved in the development of the nervous system. *Hum Mol Genet* 7: 227–237 [PubMed: 9426258]
- Yang H, Wang H, Shivalila CS, Cheng AW, Shi L. and Jaenisch R. (2013) One-step generation of mice carrying reporter and conditional alleles by CRISPR/Cas-mediated genome engineering. *Cell* 154: 1370–1379 [PubMed: 23992847]
- Yu T, Li Z, Jia Z, Clapcote SJ, Liu C, Li S, Asrar S, Pao A, Chen R, Fan N, Carattini-Rivera S, Bechard AR, Spring S, Henkelman RM, Stoica G, Matsui S, Nowak NJ, Roder JC, Chen C, Bradley A. and Yu YE (2010) A mouse model of Down syndrome trisomic for all human chromosome 21 syntenic regions. *Hum Mol Genet* 19: 2780–2791 [PubMed: 20442137]
- Yu Y. and Bradley A. (2001) Engineering chromosomal rearrangements in mice. *Nat Rev Genet* 2: 780–790 [PubMed: 11584294]
- Yuen RK, Neumann SM, Fok AK, Penaherrera MS, McFadden DE, Robinson WP and Kobor MS (2011) Extensive epigenetic reprogramming in human somatic tissues between fetus and adult. *Epigenetics & chromatin* 4: 7 [PubMed: 21545704]
- Zheng B, Sage M, Sheppard EA, Jurecic V. and Bradley A. (2000) Engineering mouse chromosomes with Cre-loxP: range, efficiency, and somatic applications. *Mol Cell Biol* 20: 648–655 [PubMed: 10611243]

Fig.1.

Diagram of the DNA duplications in partial trisomy 21 cases, mapped on the ideogram of Hsa21, related to the hypothesis of the existence of “the highly restricted Down syndrome critical region (HR-DSCR)”. The presence of a DS phenotype in DUP21SOL, PROBAND, and DUP21HAD and the absence of a DS phenotype in nsv1060057 were used to support the presence of HR-DSCR. DUP21JG and DUP21JL also exhibit a DS phenotype but the individuals also have additional genomic alterations beyond partial trisomy 21.

	Mouse Mutants	Copy #			Cognitive deficits
		D	K	X	
A. <i>Dp(16)1/+</i>					
		3	3	3	Yes
B. <i>Dp(16)1/Dyrk1a-</i>					
		2	3	3	PR
C. <i>Dp(16)1;Ms1Rhr</i>					
		2	2	2	PR
D. <i>Dp(16)1/Df(16)7</i>					
		2	2	3	NR
E. <i>Ts65Dn/Kcnj6-</i>					
		3	2	3	PR

Fig.2. Actions and interactions of the critical genes located within the *Cbr1-Fam3b* region which was converted to a single copy in *Ms1Rhr*, to normalize gene dosage in the indicated regions. C, *Cbr1*; D, *Dyrk1a*; F, *Fam3b*; K, *Kcnj6*; M, *Mx2*; X, a gene located between *Kcnj6* and *Mx2* on Mmu16. PR, partial rescue observed; NR, no rescue observed.

Fig.3.

Impacts of CRISPR/Cas9-mediated genome editing on DS-modeling. **(A)** Fibre-FISH -based characterization of the genomic regions altered via CRISPR/Cas9-mediated editing showed complex rearrangements, which include duplication, inversion, deletion and compound rearrangements. **(B)** Delivery of CRISPR/Cas9 reagents into two-cell embryos significantly enhances the efficiency of targeted knockin when compared with injection to zygotes. This is caused by open chromatin status and longer G2 stage in the two-cell stage, which increases the efficiency of homology-directed repair (HDR) over non-homologous end joining (NHEJ).

Fig.4.

Example of DS-DM and evidence for early epigenetic aging in human DS vs. control T cells. (A) Quantitative data for a locus with strong DS-DM in adult CD3+ T cells. The gene map is shown, with the DS-DM differentially methylated region (DMR) indicated. Data are from Illumina 450K methylation arrays, showing fractional methylation values for the indicated CpG (cg09981914), in a series of cases expanded since our previous publication (Mendioroz *et al.* 2015). At this locus, methylation is markedly reduced in DS compared to healthy euploid controls. Additional data from euploid adults with Type 2 diabetes (T2D)

and alopecia areata (AA) support the conclusion that the DS-DM is due to Trisomy 21 and is not secondary to associated disease processes. **(B)** Validations of DS-DM by bisulfite sequencing in two representative DS cases and two controls. The DMR encompasses multiple contiguous CpGs centered on cg09981914. **(C)** Early epigenetic aging in DS compared to control euploid human T cells. The fitted line for methylation age of the DS T cell samples in these X-Y plots has a slope that is identical to the fitted line for the euploid control T cell samples. Thus, there is no evidence of acceleration of the *rate* of aging. However, the DS line has a higher Y-axis intercept than the control line, indicating that individuals with DS have undergone *early* epigenetic aging, with the difference from controls already established prior to adulthood.

Fig.5. Mouse models for investigating mechanisms of DS-DM. Mechanistic hypotheses and questions that are being addressed using these models are indicated on the right.

Fig. 6.

DS-DM in human DS and the Ts65Dn and *Dp(16)1Yey* mouse models, assessed by whole-genome bisulfite sequencing (WGBS) of DNA from brain tissue (cerebral cortex).

Differentially methylated region (DMR) detection reveals similarities and differences between two DS mouse models [Ts65Dn and *Dp(16)1Yey*], and between each model and human DS. DMRs were defined using the DEFIANT algorithm (Condon *et al.* 2018). Gains of methylation, with very few losses, are seen in the *PCDHA-PCDHG* protocadherin gene clusters, both in human DS and in the two mouse models. However, the patterns differ.

Euploid age-matched controls were sequenced for the human data, and wild-type littermates were the controls for the mouse data. The delta methyl tracks indicate all statistically significant DMRs comparing DS vs control and mutant vs WT mouse brains; all adequately covered CpGs (>20X) are indicated in the bottom tracks. The linear scale for differences in fractional methylation is from -0.4 to +0.4, with the greatest differential methylation in this chromosomal region being +0.4.